Agrobacterium-mediated floral dip transformation of the model polyploid species Arabidopsis kamchatica

Yew, Chow-Lih ; Kakui, Hiroyuki ; Shimizu, Kentaro K

Abstract: Polyploidization has played an important role in the speciation and diversification of plant species. However, genetic analyses of polyploids are challenging because the vast majority of the model species are diploids. The allotetraploid Arabidopsis kamchatica, which originated through the hybridization of the diploid Arabidopsis halleri and Arabidopsis lyrata, is an emerging model system for studying various aspects of polyploidy. However, a transgenic method that allows the insertion of a gene of interest into A. kamchatica is still lacking. In this study, we investigated the early development of pistils in A. kamchatica and confirmed the formation of open pistils in young flower buds (stages 8–9), which is important for allowing Agrobacterium to access female reproductive tissues. We established a simple Agrobacterium-mediated floral dip transformation method to transform a gene of interest into A. kamchatica by dipping A. kamchatica inflorescences bearing many young flower buds into a 5% sucrose solution containing 0.05% Silwet L-77 and Agrobacterium harboring the gene of interest. We showed that a screenable marker comprising fluorescence-accumulating seed technology with green fluorescent protein was useful for screening the transgenic seeds of two accessions of A. kamchatica subsp. kamchatica and an accession of A. kamchatica subsp. kawasakiana.

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Electric supplementary materials

Title:

Agrobacterium*-mediated floral dip transformation of the model polyploid species *Arabidopsis kamchatica

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Table S1

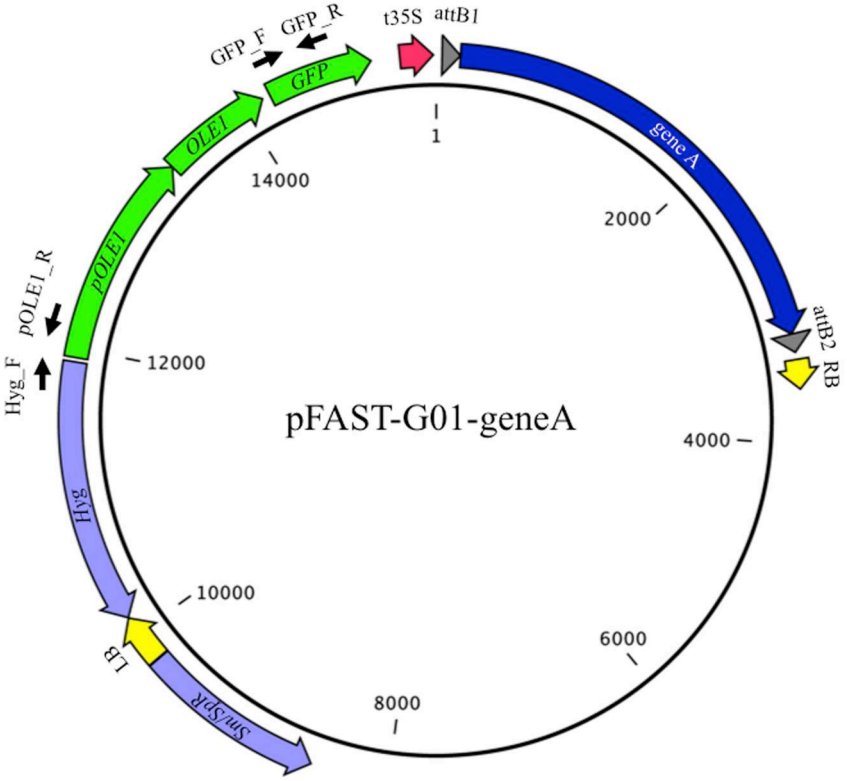

Fig. S1 Primers designed in the pFAST-G01-geneA binary vector for PCR amplification. Black arrows, specific primers designed for PCR amplification (Table S1); *OLE1*, *OLEOSIN1*; *pOLE1*, promoter of *OLE1*; *GFP*, green fluorescent protein; *Hyg*, hygromycin resistance gene; *Sm/SpR*, spectinomycin resistance gene; LB, left border; RB, right border; t35S, terminator of CaMV 35S; attB1 and attB2, Gateway recombination sequences.

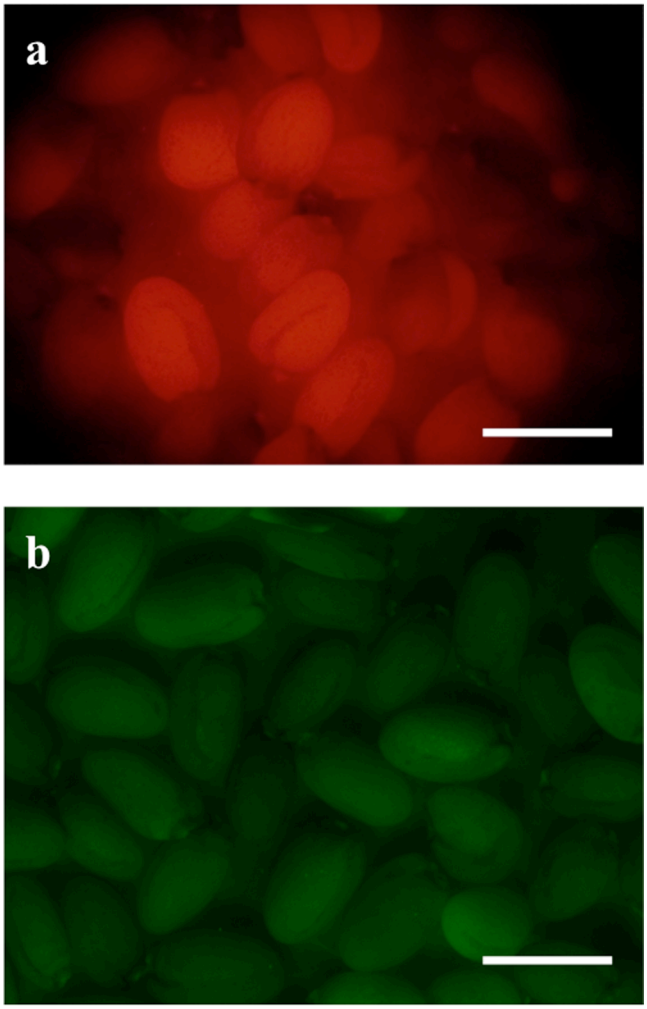

Fig. S2 Observation of seeds from the Takashima accession under a fluorescence stereomicroscope. **a** Takashima accession seeds exhibited strong autofluorescence under a fluorescence stereomicroscope equipped with an RFP filter. **b** Takashima accession seeds exhibited weak autofluorescence under a fluorescence stereomicroscope equipped with a GFP filter. Bar, 1 mm.

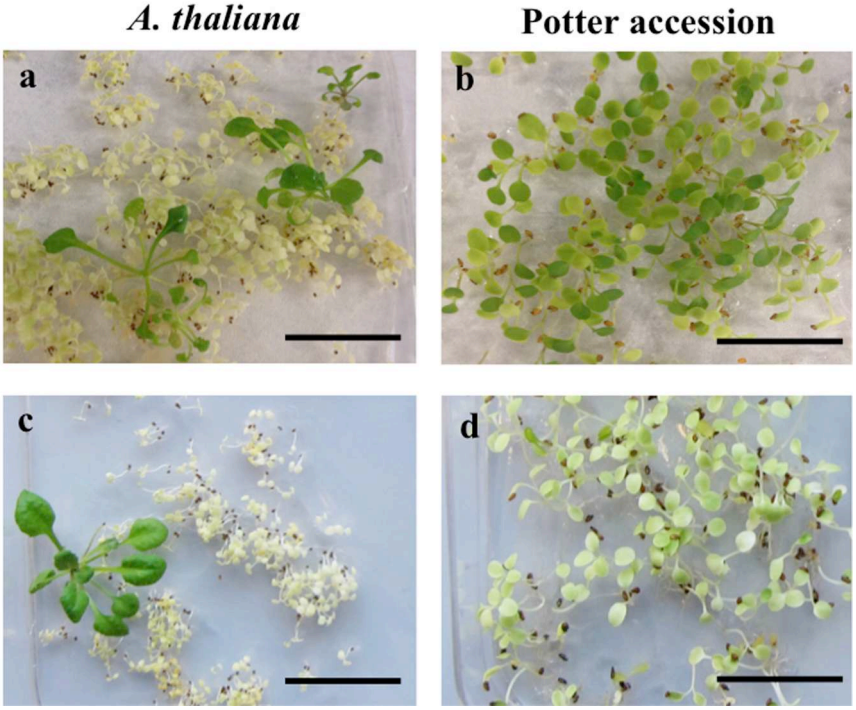

Fig. S3 Kanamycin treatment of the wild-type Potter accessions and T_1 seeds of transgenic *A. thaliana* transformed with the kanamycin resistance gene as a control. Seeds were grown on ½× MS plates containing: **a**, **b** 50 μg ml^{-1} and **c**, **d** 250 μg ml^{-1} of kanamycin. Bar, 2 cm.

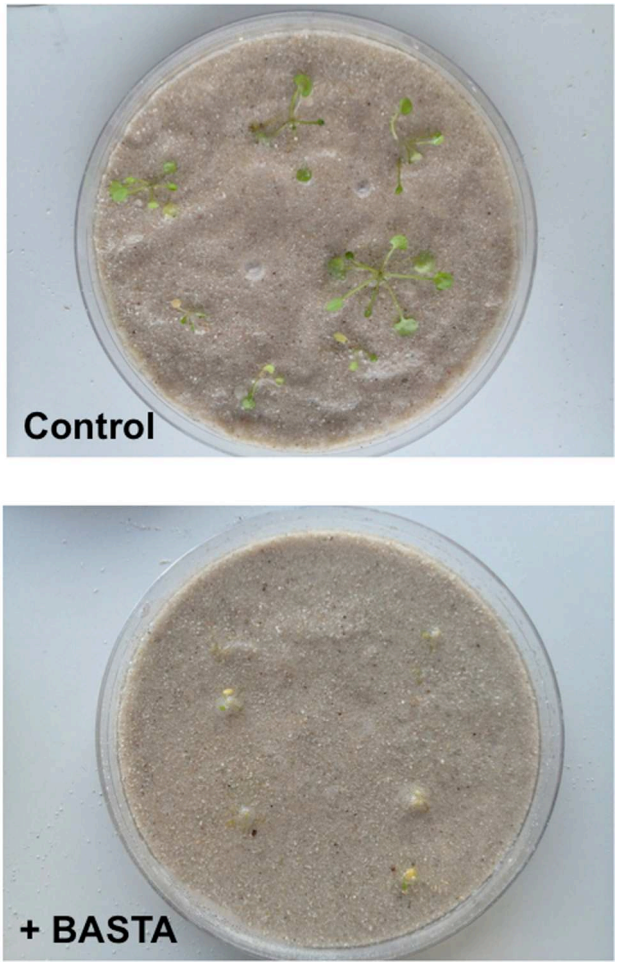

Fig. S4 Treatment with Basta for 2–3 weeks killed the Takashima accession seedlings.

Table S1 Specific primers designed in the pFAST-G01-geneA binary vector for PCR amplification

Forward primer	Sequence	Reverse primer	Sequence	Annealing temperature (°C)	Elongation time (s)
GFP_F	ACGTAAACGG CCACAAGTTC	GFP_R	TGTGGCGGATC TTGAAGTTCAC	60	30
Hyg_F	GGGGAATTTAT GGAACGTCA	pOLE1_R	CTCTAGAGGAT CCCCGGGTA	60	30